11:00

750-3 Modulation of Smooth Muscle Cell Migration/Invasion by Plasminogen Activators, Urokinase Receptor, and Low Density Lipoprotein Receptor Related Protein

Shunichiro S. Okada, Stephen R. Grobmyer, Elliot S. Barnathan. *University of Pennsylvania, Philadelphia, PA*

Smooth muscle cell (SMC) migration is an early response to vascular injury contributing to the development of intimal thickening. Upregulation of several components of the plasminogen activator (PA) system have been documented after vascular injury. We utilized a Transwell filter assay system and cultured human umbilical vein SMC to more thoroughly define the role of four different components of the PA system on SMC migration: 1) plasminogen activators, 2) plasmin, 3) PA receptors (e.g. u-PAR) and 4) PA clearance receptors (e.g. low density lipoprotein receptor related protein, LRP). Active two chain urokinase stimulated random migration (1.9 ± 3 fold increase, 0.36 nM, $p = 0.0001$) which was inhibitable by diisopropylfluorophosphate (DFP), plasminogen activator inhibitor-1 (PAI-1) or aprotinin. The aminoterminal fragment of u-PA (ATF) increased migration as well (1.4 ± 0.1 fold, $p < 0.001$) but was not inhibitable by aprotinin. Low molecular weight u-PA also enhanced migration (1.5 ± 0.3 fold, $p = 0.003$); the effect was inhibitable by aprotinin and was additive when combined with ATF. The stimulatory effect was not specific for u-PA in that t-PA also stimulated migration (1.7 ± 0.1 fold, 10 nM, $p = 0.0001$); the augmentation was inhibitable by DFP, PAI-1, or aprotinin and was additive to the u-PA effect (2.4 ± 0.4 fold, $p < 0.02$ vs. t-PA alone). Antibodies to either u-PAR or LRP inhibited migration (19 ± 9% and 43 ± 4% of control, respectively, $p = 0.0001$) but only anti-u-PAR antibodies inhibited baseline SMC invasion (41 ± 9% of control, $p = 0.01$) in a collagen gel assay. These data demonstrate the ability of several components of the PA system to modulate SMC migration and invasion in vitro via plasmin dependent and independent mechanisms.

11:15

750-4 Efficient Adenovirus-Mediated Perivascular Gene Transfer and Protein Delivery by a Transvascular Injection Catheter

Khawar Mehdi, Robert L. Wilensky, Sang Hong Baek, Bruce C. Trapnell, Keith L. March. *Krannert Institute of Cardiology, Indiana University Medical Center, Indianapolis, IN; RL Roudebush VAMC, Indianapolis, IN; Genetic Therapy, Incorporated, Gaithersburg, MD*

Intraluminal delivery catheters have successfully delivered agents to vascular tissue in vivo, but are characterized by limited efficiency, partially due to intravascular loss of agent. The kinetics of adenoviral cell attachment also dictate the need for prolonged vector exposure times to optimize transduction. The use of needle-tipped injection catheters (NIC) capable of direct perivascular injection of agents may enhance delivery efficiency as well as transduction by interstitial deposition of agent without the need for prolonged vascular occlusion. The delivery distribution, efficiency and transduction properties of NIC were evaluated *in vivo* in 18 normal porcine coronary arteries acutely following injection of ^{125}I-albumin or fluorescein-conjugated heparin; and 7 days after injection of a replication-deficient recombinant adenoviral vector encoding nuclear-targeted β-galactosidase (β-gal) as a marker gene. The fractional delivery of albumin was 0.54 ± 0.30% into the arterial wall, 0.98 ± 0.70% into the perivascular space, and 2.7 ± 2.4% into perivascular myocardium; while evaluation of the entire myocardium surrounding the target vessel confirmed presence of 15% of the total agent delivered. Fluoresceinated heparin was predominantly seen in the adventitia and perivascular tissue, and was distributed longitudinally for distances of 15–20 mm. Adenoviral gene transfer was seen with high frequency in adventitia and perivascular tissues extending up to 10 mm from the target vessel, as well as in the epicardium overlying the delivery sites, suggesting convective vector transport; but medial transduction was limited. Catheter-based transvascular injection provides for comparatively efficient agent delivery, and may represent an approach to the genetic modulation of a range of perivascular tissues without the need for prolonged vascular occlusion.

11:30

750-5 Heparin Reverses the Antiplatelet Effect of Nitric Oxide

Gilbert R. Upchurch, Jr., Jane E. Freedman, Yingyi Zhang, George N. Welch, Joseph Loscalzo. *Brigham and Women's Hospital, Boston, MA; Boston University School of Medicine, Boston, MA*

Heparin has been shown to increase thromboxane A_2 generation by platelets *in vivo*, and we have shown that expression of the platelet activation antigen P-selectin is induced by heparin. The mechanism for these adverse, prothrombotic effects of heparin remains unknown. Since endothelium-derived nitric oxide (NO) is a potent inhibitor of platelet activation, we examined the effects of heparin on the antiplatelet effects of this endothelial product. Increasing concentrations of heparin (0, 0.5, 5, or 50 U/ml) were incubated with gel-filtered platelets to which the NO-donor S-nitroso-glutathione was added and platelet aggregation responses to adenosine 5'-diphosphate determined. While incubation of platelets with heparin alone did not alter the aggregation response and incubation with 1 μM S-nitroso-glutathione inhibited the normalized extent of aggregation by 50%, the addition of heparin to S-nitroso-glutathione led to a dose-dependent increase in aggregation response (EC_{50} = 1.79 U/ml, $p = 0.03$ by ANOVA). Using the Griess reaction and the absorbance of the chromophore of acidified nitrite, we observed a concomitant dose-dependent decrease in NO with increasing heparin concentrations (IC_{50} = 7.30 U/ml, $p = 0.007$ by ANOVA). The increase in aggregation response correlated negatively with the decrease in nitrite in these experiments ($R = -0.74$). These data show that heparin can impair the antiplatelet effects of nitric oxide and suggest that one mechanism by which heparin promotes platelet activation *in vivo* is by attenuating the inhibitory effects of endothelium-derived nitric oxide.

11:45

750-6 Dual Inhibition of Neutral Endopeptidase (NEP) and Angiotensin Converting Enzyme (ACE) Suppresses Atherogenesis and Improves Endothelial Function in Hypercholesterolemic Rabbits

Iftikhar J. Kullo, Virginia M. Miller, George M. Lawson, John C. Burnett Jr.. *Mayo Clinic, Rochester, MN*

NEP and ACE are colocalized ectoenzymes which metabolize several vasoactive peptides. Combined inhibition of the two enzymes could have unique antiatherogenic effects by augmenting the antimitogenic-vasorelaxing properties of the natriuretic peptides and kinins whilst decreasing levels of angiotensin II. We investigated the effects of UK-81252 (UK) (Pfizer), a dual inhibitor of NEP and ACE, on vascular reactivity and atherogenesis in male New Zealand White rabbits fed a 1% cholesterol diet for 10 weeks. Animals were either untreated (n = 6), or treated with UK 5 mg/kg/day orally (n = 8). At 10 weeks, mean arterial blood pressure (86 ± 4 vs 81 ± 3 mmHg) and plasma cholesterol levels (1937 ± 195 vs 2022 ± 162 mg/dl) were comparable in the two groups. The percent surface area of the proximal thoracic aorta affected by atheroma (measured after Oil-Red O staining) was significantly lower in the treated group (22 ± 7% vs 48 ± 9%; $p < 0.01$). Endothelial dependent relaxations to acetylcholine, in vascular rings from the thoracic aorta, were significantly greater in the UK treated group compared to the untreated group:

	MR	EC50
Untreated	*56 ± 6.*	*6.4 ± 0.34*
Treated	86 ± 2.6**($p < 0.001$)	7.0 ± 0.03*($p < 0.02$)

MR = maximum relaxation. EC50 = concentration causing a 50% decrease of phenylphrine induced contraction and expressed as a negative logarithm of the molar concentration.

These results demonstrate that chronic dual inhibition of ACE and NEP suppresses atherogenesis and improves endothelial function in hypercholesterolemic states in the absence of any significant effects on blood pressure or plasma cholesterol.

751 Effects of Lipid Lowering Therapy

Tuesday, March 26, 1996, 10:30 a.m.–Noon
Orange County Convention Center, Room 414C

10:30

751-1 Secondary Prevention in CAD: A Significant Correlation Between LDL-Cholesterol (LDL-C) Achieved Therapeutically and Extent of Progression Reduction Claims for LDL-C Target Levels < 100 mg/dl

H.P. Bestehorn, U.F.E. Rensing, H. Roskamm, L. Benesch, G. Blümchen, P. Mathes, L. Kappenberger. *Herz-Zentrum Bad Krozingen; Fachklinik Rhein Ruhr Essen; Klin. Roderbirken Leichlingen; Klinik Höhenried; CHUV Lausanne*

The Coronary Intervention Study (CIS) is a multicenter, randomized, double-blind, placebo-controlled study to investigate the effect of lipid-lowering with simvastatin (S) on progression of coronary artery disease (CAD) in 254 men with documented CAD and hypercholesterolemia. Treatment with up to 40

mg S or placebo (P) included diet and was persued for 2.3 years. In the P-group, serum lipids remained unchanged, while the S-group showed a 35% LDL-C decrease. 205 pts (81%) had a 2nd coronary angiography and 203 (80%) were evaluable by QCA. In the S-Group, coronary progression (per patient mean change in minimum lumen diameter (ΔminD) was significantly less than in the P-Group (−0.02 mm vs −0.1 mm; p = 0.0022).

In the S group (n = 103), the individual absolute LDL-C-levels achieved therapeutically were significantly correlated with the corresponding MLD decreases (R = 0.241; p < 0.015). Dividing the correlated parameters in tertiles of their distribution curves, "progression" (mean MLD-loss of > +0.04 mm) occured in 45.4% in the upper (≥ 115 mg/dl), in 34.4% in the medium (95–114 mg/dl) and in 23.5% in the lowest LDL-C-tertile (≤ 94 mg/dl). The corresponding rates for "No Change, Regression"(mean MLD-loss ≤ +0.04 mm) were 54.6%, 68.6% and 76.5%.

Conclusion: In patients with elevated LDL-C-levels, CAD-progression can be slowed down significantly by lipid lowering with S. The extent of progression reduction is correlated with the absolute LDL-C-levels achieved therapeutically; based on the tertile-dependent progression rates, LDL-C target levels should be below 100 mg/dl.

10:45

751-2 Scandinavian Simvastatin Survival Study (4S): Cost-Effectiveness (CE) of Cholesterol Lowering Treatment

Bruce Kinosian, Henry Glick, J Sanford Schwartz for the 4S Group. *University of Pennsylvania, Philadelphia, PA*

In contrast to the demonstrated clinical benefits of cholesterol-modifying drugs in pts with CAD and moderate cholesterol elevation, less evidence is available about CE. We assessed the CE of simvastatin Rx using a simulation model integrating data from 4S.

Life expectancies were estimated from 4S survival using Weibull failure time models. Cardiovascular hospitalization and drug use were derived from 4S data. Cost and benefits were discounted 5% per yr. Medical service costs were estimated using adjusted Medicare DRG & RBRVS reimbursement (lower bound) and Medstat allowed charges (upper bound); drug costs were estimated using average wholesale price plus dispensing fees. CE analyses were performed for both the 5 yr 4S observation period and projected for lifetime Rx.

During the 4S follow-up, treated patients lived an additional 0.065 yrs (Discounted: 0.053 yrs [95% CI: 0.019–0.087]). Simvastatin cost was partially offset by reduced medical care expenditures. Net costs of Rx were $896 (Medstat) – $3,526 (Medicare) over the 5 yr 4S observation period. Projected incremental survival for lifetime Rx was 2.22 yrs (Discounted: 0.926 yrs [95% CI: 0.561–1.29]). Expected net Rx cost was $6,798 (Medstat) – $11,045 (Medicare), with cost/YOLS of $7,340–$11,927 [95% CI: $5,918–$44,617].

While the CE of simvastatin Rx in pts with CAD and moderate cholesterol intervention is within the range of accepted medical interventions when the analysis is restricted to the 5-year 4S observation period, long-term Rx is even more cost-effective, as the greatest benefits occur after the initial 5 yrs.

11:00

751-3 Atherosclerosis Plaque Regression on the Thoracic Aorta Assessed by Transesophageal Echocardiography in Patients With Dysplipidemia and Coronary Artery Disease

Miguel Zabalgoitia, William Linn, Rosario Mercado, Lori Oneschuk, Karen Vanderwoud, Sheri Y. Nottestad, John Cornell, Robert A. O'Rourke. *University of Texas Hlth Science Ctr, San Antonio, TX*

Transesophageal echocardiography (TEE) provides an excellent means to characterize atherosclerotic plaques (AP) on ascending (AA), transverse (TA), and descending aorta (DA). To determine if TEE can be used to evaluate AP modification, 10 male pts (mean age 63 yrs) with dyslipidemia and documented coronary artery disease were treated according to National Cholesterol Education Program. AP areas were planimetered at 2 cm apart along the AA, TA, and DA from biplane TEE at baseline and at 12 months of therapy. Exact distance from the incisors was indicated to assure that comparisons on the same patient were taken at the same site. Total plaque area (TPA) index was calculated by adding all areas suitable for comparison and divided by the number of entries. *Results:* When TPA index at 12 months was compared to baseline, it changed from 1.54 cm^2 to 1.15 cm^2, p < 0.01, a 25% reduction; total cholesterol changed from 209 mg/dl to 170 mg/dl, p < 0.001, a 19% reduction, low density lipoprotein (LDL-cholesterol) changed from 140 mg/dl to 109 mg/dl, p < 0.002, a 22% reduction; and total cholesterol/high density lipoprotein ratio changed from 5.6 to 4.8, p < 0.03, a 14% reduction. TPA index and LDL-cholesterol reductions correlated well r = 0.76, SEE = 0.19, p < 0.05. *Conclusion:* Agressive lipid lowering therapy results in a 25% reduction on AP of the thoracic aorta after 12 months. Reduction of LDL-cholesterol correlates well with AP regression. TEE is a useful non-invasive technique to evaluate AP modification by lipid lowering treatment.

11:15

751-4 Regression of Atheroma Seen With Intravascular Ultrasound: A Placebo Controlled Study of the Effect of Gemfibrozil on Peripheral Atherosclerosis

Andrew N. Deaner, Jeff Ball, Arzu A. Cubukcu, Simon Megary, Peter J. Scott, Paul Brooksby, Mohan Lal, Mohan Sivanathan, Gordon J. Williams. *Killingbeck Hospital, Leeds, UK*

The Killingbeck Regression of Atheroma Study (KRAS) was a double blind placebo controlled study of the effect of gemfibrozil plus lipid lowering diet versus lipid lowering diet only on atheroma in the distal aorta and iliac arteries of patients with coronary artery disease and moderately raised lipids (mean total cholesterol = 6.8 mmol/l). Serial cross-sectional images of these vessels were recorded at 2 mm intervals using a 6 French, 20 MHz intravascular ultrasound (IVUS) catheter during a controlled pull back in 39 patients (male 26, ages 38–70). Patients were given dietary advice and randomized to treatment with gemfibrozil 600 mg bd or placebo. After a mean of 371 days the 31 patients (17 in gemfibrozil group) who completed the study underwent repeat IVUS imaging. A total of 1144 segments of artery were matched according to the position of bifurcation points and measurements of cross-sectional area of atheroma made by computerized planimetry. Baseline demographics, physical and lipid data were well matched between the treatment groups. In the gemfibrozil group significant reductions compared to the diet only group, were seen in total cholesterol (9%, p = 0.03), triglycerides (55%, p = 0.0001) and LDL/HDL ratio (26%, p = 0.02), a significant increase in HDL cholesterol level (27% p = 0.05) was also seen. No overall significant change in the mean cross-sectional area of atheroma was seen in the diet only group but there was an 9.85% decrease of this measurement in the gemfibrozil group (p = 0.009). Analysis of cross-sectional plaque area changes within individual patients showed significant (p < 0.05) regression of > 5% of plaque in 5 of the gemfibrozil group but only 1 of the diet only group.

Thus, this study using repeated IVUS measurements of plaque cross-sectional area showed significant regression of peripheral atheroma in patients treated with gemfibrozil plus lipid lowering diet.

11:30

751-5 Pravastatin Reduces Transient Myocardial Ischemia in Patients With Angina Pectoris and Significant CAD

Ad J van Boven, J Wouter Jukema, Aeilko H Zwinderman, Albert VG Bruschke, Kong I Lie for the REGRESS Study. *The Interuniversity Cardiology Institute, ICIN, Utrecht; Thoraxcenter, Univ. Hospital, Groningen, The Netherlands*

Usual anti-anginal treatment, including PTCA and CABG, will relieve symptoms, but a complete abolishment of Transient Myocardial Ischemia (TMI) is not always attained. We hypothesized that pravastatin 40 mg (P) in addition to conventional therapy may reduce TMI and studied its 2 year treatment effect in 768 patients from the REGRESS study, using 48 hour ambulatory ECG's. In this placebo controlled study only men with angina pectoris, documented coronary artery disease and a cholesterol between 4 and 8 mmol/l were included. Separate randomization blocks were made from pts who underwent respectively PTCA, CABG ormedical management. During Holter monitoring anti-ischemic medication was continued. To analyze the effect of P we used a logistic regression model with random effects. During the study the percentage of patients with TMI decreased from 28% to 19% with P and increased from 19% to 23% with placebo. The odds ratio for TMI of P versus placebo was 0.62 (95% CI 0.41–0.93; p = 0.021). Total duration of ischemia and ischemic burden were reduced with 38 min and 19 mm.min with P and 9 min and 8 mm.min with placebo, p = 0.017 and 0.0058 respectively. P reduced TMI despite optimal anti-anginal treatment. These data on the anti-ischemic action of P are in line with its reduction in clinical events in REGRESS.

11:45

751-6 Lowering "Desirable" Cholesterol Levels Improves Flow-Mediated Vasoactivity in Healthy, Middle-Aged Men

Robert A. Vogel, Marv C. Corretti, Gary D. Plotnick. *University of Maryland School of Medicine, Baltimore, MD*

Current National Cholesterol Education Program guidelines consider desir-